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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/897,988	07/05/2001	Yuta Nakai	US-1420	1677
38108 7590 06/11/2010 CERMAK NAKAJIMA LLP ACS LLC 127 S. Peyton Street Suite 210 ALEXANDRIA, VA 22314				
EXAMINER				
MARVICH, MARIA				
ART UNIT		PAPER NUMBER		
1633				
NOTIFICATION DATE		DELIVERY MODE		
06/11/2010		ELECTRONIC		

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary

Application No.

09/897,988

Applicant(s)

NAKAI ET AL.

Examiner

MARIA B. MARVICH

Art Unit

1633

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --
Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 3/2/10.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 14, 19, 23, 27, 32 and 33 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☒ Claim(s) 14, 19, 23 and 27 is/are allowed.
- 6) ☒ Claim(s) 32 and 33 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☒ The drawing(s) filed on 11 August 2003 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.
- Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☒ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- 1) ☐ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☐ Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date _____
- 4) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date _____
- 5) ☐ Notice of Informal Patent Application
- 6) ☐ Other: _____

DETAILED ACTION

This office action is in to an amendment filed 3/4/10. Claims 1, 6 and 12-17 are pending.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 1, 6, 7 and 11-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kojima et al (US 5,830,716; see entire document) in view of Calhoun et al (J Bacteriol. 1993 May; 175(10): 3020-3025; see entire document), Ciccognani et al (FEMS Microbiology Letters 94, 1992, page 1-6; see entire document) or Kusomoto et al (Arch Microbiol, 2000, Vol 173, pages 390-397; see entire document) or Sone et al (Collection of Summaries of Lectures made at the Meeting of Japan Bioengineering Association, September 15, 1995, p.10). **This rejection is maintained for reasons of record in the office action mailed 12/4/09 and restated below.**

Kojima et al provide methods of using bacteria for production of amino acids. Cells are grown in culture wherein the target substance is produced in the culture medium and isolated thereof (see e.g. abstract). Specifically, Kojima et al teach that *E. coli* and *Coryneform* bacteria are well known in production methods of threonine, lysine and phenylalanine wherein the cells are engineered to improve production by altering a biochemical cellular pathway (see e.g. col 3, line 55-col 4, line 35).

Kojima et al description of altered biochemical pathways does not include one wherein the high energy efficiency pathways (*nuo* and cytochrome bo) and low efficiency pathway (*ndh* and cytochrome bd) are altered.

However, **Kusomoto** et al teaches that cells used for production methods of amino acids can be altered for improved amino acid production by altering the aerobic metabolism of the cell. Specifically, by deleting the low efficiency gene.

"In order to improve the efficiency of cell growth and amino acid production, it is important to understand the aerobic energy metabolism or, more specifically, the respiratory proton pumps in the bacterium."

"Cytochrome *bd*-type oxidase has been shown to have a lower H₂/O ratio than haem-copper oxidases (Miller and Gennis 1985; Puustinen et al. 1991). It has been reported that the H₂/e- ratio is about 1 for intact cells of *C. glutamicum* with endogenous substrate. This is lower than that expected if an *aox*-type haem-copper oxidase is operating (Kawahara et al. 1988). Thus, it is likely that deletion of the cytochrome *bd* genes would increase the H₂/e- ratio of the respiratory chain, the efficiency of energy metabolism, and consequently the growth yield of the bacterium."

Hence, Kusomoto et al directly link increased amino acid production with the growth yield of the cell and the energy efficiency of the cell.

It is established in the art that the energy efficient pathways of a number of microorganisms comprise high and low efficiency pathways and that alteration of the pathways can increase the energy efficiency of the cells and alter the growth yield. High energy efficiency pathways include *nuo* which encodes NHD-I and cytochrome bo and low efficiency are *ndh* which encodes NDH-II and cytochrome bd.

For example, **Calhoun** et al teach

"In principle, by directing the electron flux through specific respiratory components, the energetic efficiency of the *E. coli* respiratory chain can be varied between 4H⁺/e- (with NDH-1 and the bo-type oxidase) and 1H⁺/e- (with NDH-2 and the bd-type oxidase). Since the wild-type strain contains both NADH dehydrogenases and both terminal oxidases, the value for the H⁺/e- ratio must fall between these two extremes and will vary with growth conditions."

Calhoun et al assesses growth efficiency by constructing strains to use only one NADH dehydrogenase and one terminal oxidase and determines,

“the data confirm the following expectations based on the in vitro proton translocation measurements: (i) based on the in vitro proton translocation measurements: (i) the elimination of the uncoupled NDH-2 results in increased energetic efficiency; (ii) strains that utilize the bd-type oxidase have a less-efficient respiratory chain than those using the bo-type oxidase.”

Hence, Calhoun explicitly teaches that to increase growth efficiency, one would eliminate NDH-II or bd and increase bo. Results from *nuo* are not demonstrated because at the time of publication it had not been cloned. This reference nonetheless directs one to create the strains recited in claims 1, 6, 7 and 11.

Specifically, Calhoun et al explicitly and inherently teaches strains in which bo is increased, ndh II is decreased by gene disruption or both bo is increased and ndhII is deficient. Explicitly, Calhoun teaches that strains with deleted ndh-II has been created and produces cells with increased growth yield. (These strains are encompassed by Sone et al who teaches that strains comprising bo cytochrome oxidase activity and that lack bd cytochrome oxidase activity has enhanced growth yield in *E. coli* (see Results).

By directing one to create cells with increased bo, Calhoun et al inherently directs one to increase copy number of bo. For example,

Ciccognani et al teach methods of culturing *E. coli* (RG145), which is a genetic recombinant strain in which an enzyme of the high-energy efficiency pathway was enhanced and an enzyme of low-energy efficiency was deficient. The cells contain a chromosomal deletion resulting in the inability of the cell to express *cydA* and contain a cosmid containing the *cyo* operon resulting in over expression of the cytochrome bo complex (page 2, section 3.1).

In *KSR International Co. v. Teleflex Inc.*, 82 USPQ2d 1385 (U.S. 2007), the Supreme Court particularly emphasized "the need for caution in granting a patent based on a combination of elements found in the prior art," (Id. At 1395) and discussed circumstances in which a patent might be determined to be obvious. Importantly, the Supreme Court reaffirmed principles based on its precedent that obviousness in part is predicated on use of particular known techniques that are recognized as part of the ordinary capabilities of one skilled in the art.

In the instant case, it is accepted that production methods of amino acids utilize *E. coli* and *coryneform* bacteria in which the cells are cultured and the produced amino acids are excreted and isolated from the cell culture and that these methods can be improved with predictable results by applying known techniques of cellular engineering for improved energy efficiency.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use microorganisms that have been altered to have enhanced high efficiency pathways and/or deficient low energy pathways given that these modifications are taught by multiple sources such as Calhoun et al, Ciccognani et al, Sone et al or Kusomoto et al with the known methods of producing amino acids using the methods reviewed by Kojima et al because Kojima et al teach that it is within the ordinary skill of the art to use *E. coli* to produce amino acids wherein the method requires culturing of and isolation from the culture of amino acids and because Calhoun et al, Ciccognani et al, Kusomoto et al, and Sone et al teach that production cells can be improved by altering the energy efficiency pathways of the cells and more specifically Kusomoto et al teach that the increase in energy efficiency as well as in improved growth yield is attributed to an improvement in the amino acid production in such strains. Based

upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Response to Applicants' Arguments

Applicants' arguments filed 3/4/10 have been considered but are not persuasive for the following reasons. First, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). In this case, applicants have attacked Kojima for lack of discussion of high energy and low energy efficiency pathways, Calhoun for not teaching that increasing cell growth correlates with increased amino acid production, Sone, Spehr and Ciccognani as being limited to analysis based upon alteration of high/low energy efficiency pathways that are not related to L-amino acid production and Kusimoto for lack of teaching the relationship of bo enzyme to L-amino acid production. Rather, the obviousness of a method of producing an L-amino acid by culturing a bacterium capable of accumulating the L-amino acid in the medium and furthermore comprising enhanced bo-type oxidase activity is based upon the skill of one in the art and the predictability of the *combination of events*. Specifically, *the combination of references* established that it is routine to use bacterium capable of producing L-amino acids for collection from the medium and furthermore that these bacterium can be "engineered" for improved production. Engineered bacterium wherein the energy efficiency of the cell is altered are replete in the art and clearly would be routine to produce. Calhoun et al establishes that increasing bo and decreasing ndhH (which overlap the teachings of Sone et al) allow one to interplay the energy efficiencies of a cell

and to this end create cells that are more energy efficient and have increased growth yield.

Ciccognani et al teach methods of increasing bo copy number as well as expression. Hence, the question is would it have been obvious to improve Kojima et al by producing an engineered cell with improved energy efficiency.

Kusomoto et al provide a correlation between the improved growth yields of cells achieved by altering the energy efficiency. To this end applicants appear to argue that there is no support in the references provided for such a statement. However, from Kusomoto et al,

“*Corneybacterium glutamicum* is an aerobic, gram-positive high-G+C bacterium that is of industrial importance in producing amino acids used as nutritious additives to food and feed. In order to improve the efficiency of cell growth and amino acid production, it is important to understand the aerobic energy metabolism or, more specifically, the respiratory proton pumps in the bacterium.”

“Cytochrome *bd*-type oxidase has been shown to have a lower H₂/O ratio than haem-copper oxidases (Miller and Gennis 1985; Puustinen et al. 1991). It has been reported that the H₂/e⁻ ratio is about 1 for intact cells of *C. glutamicum* with endogenous substrate. This is lower than that expected if an *aa3*-type haem-copper oxidase is operating (Kawahara et al. 1988). Thus, it is likely that deletion of the cytochrome *bd* genes would increase the H₂/e⁻ ratio of the respiratory chain, the efficiency of energy metabolism, and consequently the growth yield of the bacterium.”

Hence, Kusomoto et al directly link alteration of the energy efficiency of the cell with metabolism (amino acid production) and growth yield. The conclusion of the Japanese Examiner reflects what a person of skill in the art would conclude and that is that that these modifications improve metabolism, amino acid production by altering the growth yield and energy efficiency of the cell. While applicants indicate that this conclusion is contraindicated by the art, this argument is not supported by evidence and hence serves as objective evidence only.

Secondly, all of the instant steps are available in the art and applicable together under the principles of KSR.

If a person of ordinary skill can implement a predictable variation, § 103 likely bars its patentability. For the same reason, if a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill. Id. at ,82 USPQ2d at 1396.

When considering obviousness of a combination of known elements, the operative question is thus "whether the improvement is more than the predictable use of prior art elements according to their established functions." *Id.* at, 82 USPQ2d at 1396.

In this case, Kojima teaches that methods of producing and collecting L-amino acids from the cell are routine in the art and direct one to engineer cells in order to improve such methods. As well, the art teaches that improved conditions include those that improve high energy efficiency pathways in the cell which the art also demonstrates includes a number of such alterations which are also routine in the art. In other words, the principle of improving growth yield by improving energy efficiency of the cell has been established and the steps of exploiting such a cell to improve amino acid production as directed by Kojima and Kusomoto et al does not appear to be an inventive step. Rather applying known techniques of engineering cells for improved bo expression and decreased ndhII to known techniques is an interchangeable product wherein an improvement in the method of producing L-amino acid collection would occur without reasonable doubt.

Conclusion

No claims allowed

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after

the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MARIA B. MARVICH whose telephone number is (571)272-0774. The examiner can normally be reached on M-F (7:00-4:00).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach, PhD can be reached on (571)-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Maria B Marvich/
Primary Examiner, Art Unit 1633